

Photoperiodic Responses of the Linden Bug, *Pyrrhocoris apterus*, under Conditions of Constant Temperature and under Thermoperiodic Conditions

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ABSTRACT—Photoperiodic responses were examined in the linden bug, *Pyrrhocoris apterus* (L.) (Heteroptera, Pyrrhocoridae), from Belgorod, Russia, under conditions of constant temperature and under thermoperiodic conditions with a 12-hr cryophase and a 12-hr thermophase. The critical daylength for the induction of adult diapause was longer at lower mean temperatures. The induction of diapause under thermoperiodic conditions was determined principally by the mean temperature of the thermoperiod, although thermoperiodic conditions enhanced the induction of diapause somewhat in the threshold zone. Development under thermoperiodic conditions was more rapid than at constant temperatures that were equivalent to the mean temperature under thermoperiodic conditions. Developmental retardation was observed under photophases a little shorter than the critical value for the induction of diapause, both at constant temperatures and under thermoperiodic conditions.

INTRODUCTION

Insects in the wild develop under conditions of daily and seasonal fluctuations in temperature and these fluctuations, together with changes in the photoperiod, regulate the seasonal life cycles of insects [7, 8, 21]. In some insects, the thermoperiod has been shown to influence the parameters of photoperiodic responses by amplifying or reducing the tendency to enter diapause, or by changing the critical daylength [2]. We must consider this influence in discussions of seasonal adaptation in insects, in particular in species that live in a continental climate with large daily changes in temperature. However, photoperiodic responses of insects have usually been studied at constant temperature in the laboratory.

The linden bug, *Pyrrhocoris apterus*, has an adult diapause that is controlled by a long-day photoperiodic response [12, 18, 22]. Saunders [18] showed that photoperiod also affects the develop-

mental rate. Hodek [13] described the induction of diapause by brief exposure (2–4 days) to low temperatures in *P. apterus*. Furthermore, Honěk [14] reported that the fecundity of this species is higher under natural alternating temperatures than at a constant temperature in the laboratory. However, no experiments under thermoperiodic conditions have been performed to examine the photoperiodic response in *P. apterus*. In this study, we examined the photoperiodic response in *P. apterus*, from an inland region of Russia, at constant temperatures and under thermoperiodic conditions, and we discuss the effects of temperature on this response.

MATERIALS AND METHODS

Adults of *P. apterus*, after overwintering, were collected from the field in the reservation "Forest on the River Vorskla" (50°N, 36°E) which is located in the forest-steppe zone in the Belgorod Region, Russia. Their eggs were used for the experiments. The insects were reared in the laboratory by the method by Schlagbauer [19].

Nymphs were reared in 500-ml glass bottles. The density of the nymphs was kept at 100–150 for early instars and was reduced to 50–75 for the final (fifth) instar. Seeds of the linden tree *Tilia cordata*, strewn on the ground were collected and served as food. Puparia of the blue meat fly, *Calliphora vicina* were also supplied to late-instar nymphs and adults. Water was provided in test tubes with cotton plugs. Food and water were replaced every two or three days. Zigzag-folded strips of filter paper were used to increase the inner surface area. After adult emergence, the insects were kept in male and female pairs in Petri dishes. Thirty days after adult emergence, the females that had not laid eggs were dissected. The stage of their ovaries was examined, and the females with immature ovaries were judged as being in diapause [12].

Experiments were performed in boxes in which both temperature and photoperiod were controlled [5]. The light intensity in the boxes ranged between 180 and 250 lx and was supplied by 20 W fluorescent lamps. The deviation from the set temperatures was not more than 1.0°C. The humidity fluctuated between 50 and 70%. Photoperiodic conditions from a photoperiod with a 12-hr photophase and a 12-hr scotophase (12L-12D) to 18L-6D were used; the longest photophase corresponds to the maximum daylength in the region in which insects were collected, including civil twilight.

A square-wave type of thermoperiod was made by transferring the rearing bottles between temperatures at 9:00 and 21:00 every day. Thus, the thermoperiod was composed of a 12-hr cryo-

phase and a 12-hr thermophase (12C-12T). The thermophase coincided with the photophase, and the cryophase with the scotophase. Under 12L-12D the thermoperiod and photoperiod were completely synchronized, whereas longer photophases extended into the cryophase (Fig. 1).

RESULTS

Induction of diapause at constant temperatures

First, we compared the photoperiodic response

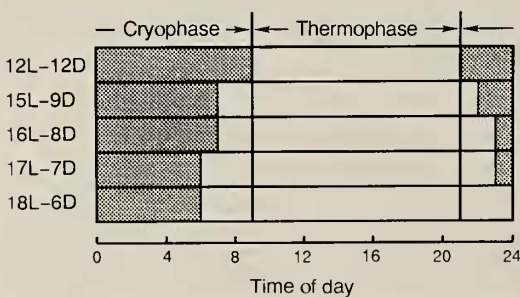


FIG. 1. The combinations of thermoperiod and photoperiod used in the experiments. Shaded bars, scotophase; open bars, photophase, in 24-hr cycles.

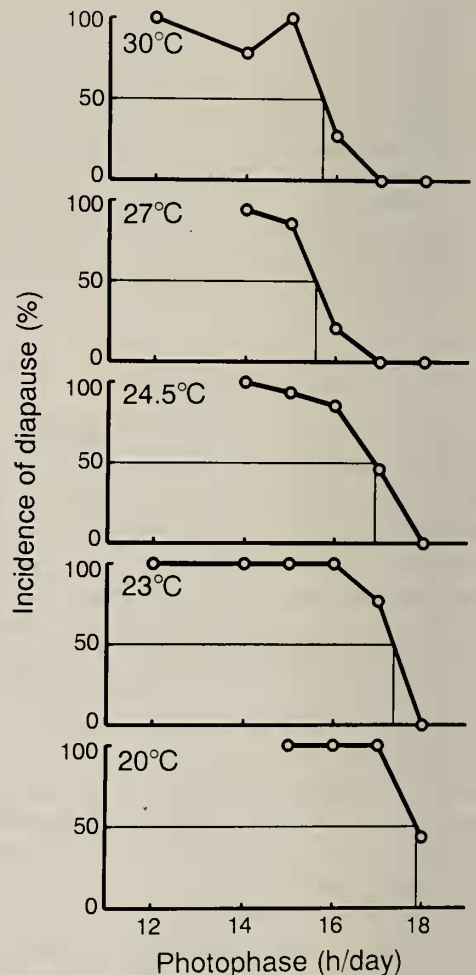


FIG. 2. The photoperiodic response curves for the induction of adult diapause at various constant temperatures in *Pyrhocoris apterus*. $n=20-32$ (20°C), $n=7-61$ (23°C), $n=154-190$ (24.5°C), $n=17-36$ (27°C), $n=14-39$ (30°C).

with respect to induction of diapause for five constant temperatures: 20, 23, 24.5, 27 and 30°C. At each temperature, the insects showed a long-day photoperiodic response. As the temperature was raised from 20°C to 27°C, the critical daylength was shortened from about 18 hr to about 15.5 hr. However, the critical daylength was about 15.5 hr both at 27°C and at 30°C, even though at 30°C some females laid eggs even under 14L-10D (Fig. 2).

Induction of diapause under thermoperiodic conditions

Next, we examined the photoperiodic response under three sets of thermoperiodic conditions: a thermoperiod with a 13°C cryophase and a 27°C thermophase (abbreviated as 13-27°C; mean,

20°C); 13-33°C (mean, 23°C); and 20-32°C (mean, 26°C). The former two conditions are similar to the natural temperatures in the summer days in the native habitat of *P. apterus*. Under 13-27°C conditions, all adults entered diapause with any photoperiod other than 18L-6D, as they do at a constant temperature of 20°C, which is equivalent to the mean temperature under the thermoperiodic conditions (Fig. 3). However, under 18L-6D, the proportion of adults in diapause was significantly higher under 13-27°C conditions than at a constant 20°C ($P < 0.05$ by Fisher's exact probability test). Furthermore, the preoviposition period was significantly shorter in nondiapause females reared under 13-27°C conditions ($n=8$, median=18, range=14-24 days) than in those reared at a constant 20°C ($n=12$, median=23, range=17-34 days; $P < 0.01$ by Mann-Whitney U test, $U=13.0$). The photoperiodic response curve under 13-33°C conditions coincided with that at a constant 23°C, which is equivalent to the mean temperature under the thermoperiodic conditions. The proportion of diapause adults in 17L-7D was a little higher under 13-33°C conditions than at a constant 23°C (Fig. 3), but the difference was not significant ($P > 0.3$ by Fisher's exact probability test). Although we did not examine the photoperiodic response at a constant 26°C, which is equivalent to the mean temperature of 20-32°C conditions, the insects showed an intermediate response between constant temperatures of 24.5°C and 27°C (Fig. 3).

Thus, photoperiodic response curves for induction of diapause under thermoperiodic conditions were similar to those obtained at constant temperatures equal to the mean temperatures of the thermoperiods, although there was a little difference under 18L-6D. Next we examined the induction of diapause in 18L-6D under six other thermoperiodic conditions: 13-24.5°C, 15-27°C, 15-33°C, 20-27°C, 20-33°C and 27-33°C. At a cryophase temperature of 13°C, the number of diapause adults varied from 5.7 to 100% of the total depending on the temperature of the thermophase. At a cryophase temperature of 15°C the percentage of diapause adults varied from 0 to 56.5%, depending again on the temperature of the thermophase. At a thermophase temperature of

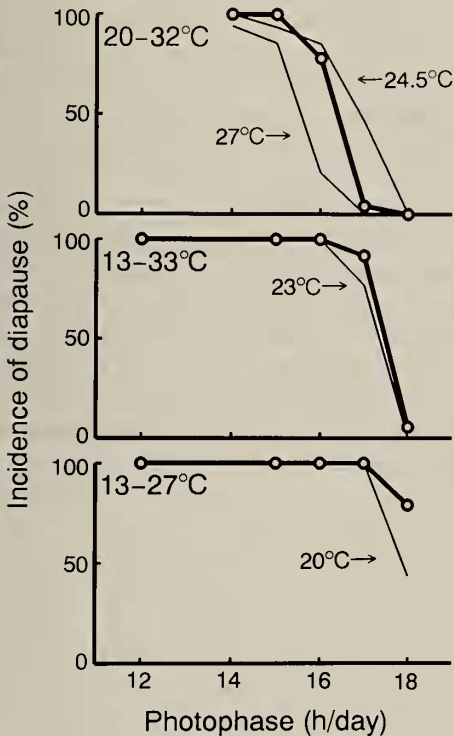


FIG. 3. The photoperiodic response curves for the induction of adult diapause under thermoperiodic conditions (12C-12T) in *Pyrrhocoris apterus*. $n=31-56$ (13-27°C), $n=37-53$ (13-33°C), $n=13-24$ (20-32°C). The photoperiodic response curves at constant temperatures equivalent to the means of the thermoperiodic conditions are shown by thin lines.

TABLE 1. Effects of thermophase and cryophase temperatures on the induction of adult diapause under 18L-6D and 12C-12T* in *Pyrrhocoris apterus*

Cryophase	Temperature (°C)		Incidence of diapause (%)	n
	Thermophase	Mean		
13	24.5	18.75	100	21
13	27	20	78.9	38
13	33	23	5.7	53
15	27	21	56.5	23
15	33	24	0	15
20	27	23.5	0	22
20	32	26	0	13
20	33	26.5	0	15
27	33	30	0	19

* See text for details.

27°C, the percentage of diapause adults varied from 0 to 78.9% depending on the temperature of the cryophase (Table 1). Therefore, neither the temperature of the cryophase nor that of the thermophase independently defined the photoperiodic response. By contrast, the insects entered diapause at lower mean temperatures and failed to do so at higher mean temperatures both at constant temperatures and under thermoperiodic conditions. However, the threshold temperature was about 21°C under thermoperiodic conditions, and was a little higher than at constant temperatures (Fig. 4).

From the results at constant temperatures and under thermoperiodic conditions, we can conclude that the mean of the day and night temperatures

defines for the most part the parameters of the photoperiodic response in *P. apterus*, although thermoperiodic conditions enhanced the induction of diapause to a slight extent near the critical daylength.

Developmental rate

Then we compared the duration of egg and nymphal stages under various photoperiodic conditions at three constant temperatures (20, 24.5, 27°C) and under two sets of thermoperiodic conditions (13–27°C, 13–33°C). Under all conditions, the females developed a little more rapidly than the males, although the results are combined in Figure 5. Both at constant temperatures and under thermoperiodic conditions, retardation of development took place near the critical daylength for the induction of diapause. The insects developed most slowly under a photophase that was a little shorter than the critical value under all temperature conditions (Fig. 5). Apart from the developmental retardation in the threshold zone, the insects developed much more rapidly under 13–27°C conditions than at a constant 20°C, and more rapidly under 13–33°C conditions than at a constant 24.5°C (Fig. 5). Thus, development under thermoperiodic conditions was much more rapid than that estimated from the mean temperature of the thermoperiod. Figure 6 shows the developmental rate, designated as the reciprocal of the duration of egg and nymphal stages, under

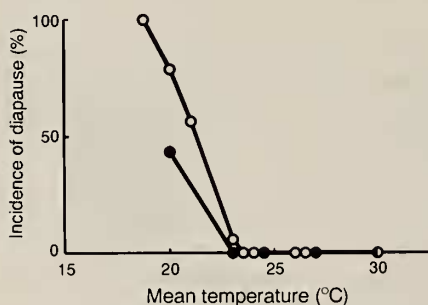


FIG. 4. Induction of adult diapause under 18L-6D in *Pyrrhocoris apterus* at various constant temperatures (solid circles) and under thermoperiodic conditions (12C-12T) (open circles).

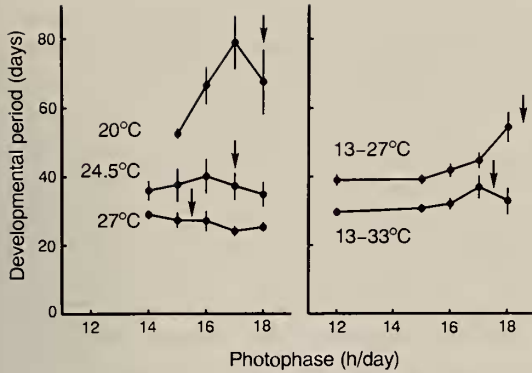


FIG. 5. Effects of photophase on the developmental period in *Pyrrhocoris apterus* at various constant temperatures and under thermoperiodic conditions (12C-12T). Solid circles with vertical lines, duration of the period from oviposition to adult emergence (mean \pm S.D., $n=31-112$). Arrows, photoperiods that induced adult diapause in 50% of insects.

15L-9D, which was not very much affected by the developmental retardation in the threshold zone. In Figure 6, we show the simple linear regression lines for the developmental rate at constant temperatures and under thermoperiodic conditions. The lower threshold temperature for development, shown as the intercept on the abscissa, was 12.5°C and 9.0°C at constant temperatures and under thermoperiodic conditions, respectively. However, there was only a slight difference in the accumulation of heat required for complete development, which is indicated by the reciprocal of the slope. It was 416 and 429 degree-days at

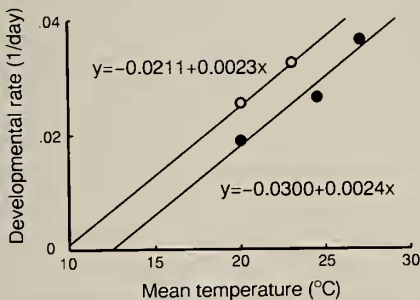


FIG. 6. Developmental rate per day under 15L-9D in *Pyrrhocoris apterus* at various constant temperatures (solid circles) and under thermoperiodic conditions (12C-12T) (open circles). Lines, simple linear regressions.

constant temperatures and under thermoperiodic conditions, respectively (Fig. 6). Thus, under thermoperiodic conditions the insects developed as rapidly as at a constant temperature that was higher by about 3.5°C than the mean temperature under the thermoperiodic conditions.

DISCUSSION

In *P. apterus* from Belgorod, the photoperiodic response for the induction of adult diapause depended on temperature (Fig. 2). At lower temperatures, the critical daylength was longer, as it is in many other insects with a long-day photoperiodic response [7, 8, 21]. However, increasing the temperature above 27°C had no effect on the critical daylength (Fig. 2). The critical daylength was found to be about 15 hr both at 20°C and at 25°C in *P. apterus* from Erevan, Armenia (40°N) [22]. Thus the dependence on temperature of the critical daylength varies between geographic populations within a species.

In many insects with a long-day photoperiodic response, thermoperiodic conditions enhance the photoperiodic induction of diapause under the natural phase relationship, i.e., when the cryophase is concurrent with the scotophase and the thermophase coincides with the photophase [1, 4, 6, 9, 10]. These results have been explained by the hypothesis that scotophase temperatures strongly influence the induction of diapause [2, 8, 10, 21]. However, experimental results obtained in recent years indicate that scotophase temperatures do not always determine the induction of diapause. For example, in the predatory bug *Podisus maculiventris* (Heteroptera, Pentatomidae, Asopinae), thermoperiodic conditions with a cool scotophase slightly reduce the incidence of diapause under short-day conditions but do not affect the critical daylength [11]. In another species in the same subfamily, *Perillus bioculatus*, thermoperiodic conditions with a cool scotophase reduce the incidence of diapause under near-critical photoperiods and, consequently, the critical daylength becomes shorter and equivalent to that at the temperature of the photophase [23]. Here we provide another example to contradict the view that the scotophase temperature has stronger effects on the induction

of diapause than the photophase temperature [2, 8, 10, 21].

In *P. apterus*, diapause under thermoperiodic conditions was determined principally by the mean temperature of the thermoperiodic conditions, although such conditions increased the incidence of diapause only slightly in the threshold zone (Figs. 3, 4). This weak enhancement of diapause induction in the threshold zone may be due to the low temperature in the scotophase as shown in other species [1, 4, 6, 9, 10]. However, we cannot deny the possibility that the 12C-12T thermoperiod itself showed a short-day effect because in the present experiments the duration of thermophase did not coincide with that of photophase except 12L-12D (Fig. 1).

P. apterus developed under thermoperiodic conditions much more rapidly than at constant temperatures equivalent to the mean temperatures of the respective thermoperiods (Fig. 4). This response does not agree with the classic law of temperature summation but has been demonstrated in some other insects [2]. With respect to the rate of the development, thermoperiodic conditions corresponded to a higher temperature than the mean value, in contrast to the induction of diapause. In the European corn borer *Ostrinia nubilalis*, in which the effect of thermoperiodic conditions has been studied most intensively, thermoperiodic conditions produce essentially the same rate of larval development as do the appropriate mean temperatures [3, 17], although a low temperature in the scotophase increases the incidence of diapause [1, 4]. Thus, in both species, day and night temperatures are integrated in different ways for the development and for the modification of the photoperiodic response.

Saunders [18] reported that *P. apterus* from Prague, Czechoslovakia (50°N), developed more slowly around the critical photoperiod for the induction of diapause than under short-day or long-day conditions at a constant temperature of 25°C. Our present experiments confirmed these findings not only at various constant temperatures but also under thermoperiodic conditions. Furthermore, the peak of retarded development moved along the photoperiodic axis in a temperature-dependent manner, together with the critical

daylength for the induction of diapause. Consequently, insects developed most slowly under a photophase a little shorter than the critical value for the induction of diapause under all temperature conditions (Fig. 5). Saunders [18] suggested that this developmental retardation promotes the seasonal synchronism in the univoltine population of *P. apterus* in Prague. Kidokoro and Masaki [16] showed a similar retardation of nymphal development near the critical photoperiod for the induction of egg diapause in both univoltine and bivoltine populations of the band-legged ground cricket, *Dianemobius nigrofasciatus*, and they attributed the main function of this response to a fine tuning of the growth rate so that the diapause stage would be reached at the appropriate time. Our field observations indicate that the Belgorod population of *P. apterus*, which lives at the same latitude as Prague, produces two generations, at least in warmer years (unpublished). Even in Czechoslovakia, *P. apterus* is not always univoltine [15, 20]. The developmental retardation in *P. apterus* must have an ecological significance even in bivoltine life cycles, as in *D. nigrofasciatus*.

Development under thermoperiodic conditions was more rapid than that estimated from the mean temperature of the thermoperiod (Figs. 5, 6). Furthermore, nymphs of *P. apterus* select warmer habitats and, therefore, their body temperature is sometimes much higher than the air temperature [15]. Therefore, under natural conditions, *P. apterus* may develop much more rapidly than in experiments at constant temperatures that were equivalent to the mean air temperatures. It is not appropriate to use the developmental parameters obtained from experiments at constant temperatures for predicting the seasonal development under natural conditions in *P. apterus*.

Furthermore, in *P. apterus* photoperiodic response for the induction of diapause depended much on temperature (Fig. 2), and the temperature is variable from year to year in Belgorod region. Therefore it is difficult to discuss the seasonal adaptation of this local population based only on the present results. We now plan to make further observations in the field and to perform experiments under natural conditions in an effort to determine the ecological significance of the

effect of photoperiod and temperature on diapause and development in *P. apterus*.

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